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EXAMINER	
THOMAS, DAVID C	

ART UNIT	PAPER NUMBER
1637	

NOTIFICATION DATE	DELIVERY MODE
03/01/2011	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

LifetechDocket@system.foundationip.com
paralegals@system.foundationip.com

Office Action Summary	Application No.	Applicant(s)	
	10/723,520	ANDERSEN ET AL.	
	Examiner	Art Unit	
	DAVID C. THOMAS	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 January 2011*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *2,3,5,6,8-18,22,23 and 43-45* is/are pending in the application.

4a) Of the above claim(s) *10-18* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *2,3,5,6,8,9,22,23 and 43-45* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's amendment filed January 6, 2011 is acknowledged. Claim 45 (currently amended) and claims 2, 3, 5, 6, 8, 9, 22, 23, 43 and 44 (previously presented) will be examined on the merits. Claims 10-18 were previously withdrawn and claims 1, 4, 7, 19-21 and 24-42 were previously canceled.

Claim Rejections - 35 USC § 103

2. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

3. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

4. Claims 5, 6, 8, 9, 22, 23, 44 and 45 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer (U.S. Patent No. 6,472,156) in view of Willey et al. (U.S. Patent Pub. No. 2003/0186246).

With regard to claims 45, 2, 23 and 23, Wittwer teaches a method for analyzing a sample or plurality of samples for the presence of one or more polynucleotide sequences of interest (a method is provided for analyzing sequence variations in nucleic acid samples comprising multiple loci, see Abstract and column 3, lines 41-45), comprising:

(i) amplifying at least one of said polynucleotides of interest (the nucleic acid samples are analyzed by performing at least two amplification reactions comprising multiple loci, column 4, lines 58-63) in the presence of:

(a) a plurality of different amplification primer pairs suitable for amplifying said polynucleotide sequences of interest (the nucleic acid samples comprising at least two or at least three loci are contacted with at least two or at least three PCR primer pairs, each pair specific to one locus under conditions which allow formation of an amplification product for each primer, column 14, line 64 to column 15, line 6); and

(b) a plurality of oligonucleotide probes, wherein each of said plurality of oligonucleotide probes is complementary to a region of a different polynucleotide sequence of interest amplified by said plurality of primer pairs and comprises a label suitable for monitoring amplification as a function of time (each loci specific amplification product is contacted with FRET labeled oligonucleotide probes that hybridize with the amplification product at an allelic sequence present in the locus, column 15, lines 15-21; FRET is measured upon hybridization of the probe within close proximity of another member of a FRET pair, which may be a labeled primer, column 11, line 66 to column 12, line 5 and column 15, lines 53-60; fluorescence emission is monitored continuously

through repeated cycles during PCR, as well as within each cycle, column 17, lines 1-8).

With regard to claim 5, Wittwer teaches a method in which the polymerase chain reaction of step (i) is carried out for a number of cycles such that the amplification remains in the linear range (PCR may be performed for up to 40 cycles, with linear linear increases of fluorescence noted, particularly with exonuclease probes, column 7, lines 7-21, column 25, lines 42-52 and Figure 2).

With regard to claim 6, Wittwer teaches a method in which the amplification in step (i) is achieved with a thermostable DNA polymerase (PCR is performed using a thermostable DNA polymerase such as Taq DNA polymerase or KlenTaqI polymerase, column 1, line 65 to column 2, line 3 and column 25, lines 42-52).

With regard to claim 8, Wittwer teaches a method in which the label is a fluorophore (probes comprise a member of a FRET pair, either a donor or acceptor moiety, column 3, lines 28-60 and column 11, lines 15-20).

With regard to claim 9, Wittwer teaches a method in which said plurality of oligonucleotide probes is selected from the group consisting of 5'-exonuclease probes, stem-loop beacon probes and stemless beacon probes (probes used for detection of PCR products include Molecular Beacons and exonuclease probes, column 3, lines 14-17 and column 7, lines 7-21).

With regard to claim 44, Wittwer teaches a method in which the amplifying the at least one polynucleotide sequences comprises as many as fourteen PCR cycles (PCR may be performed for up to 40 cycles, column 25, lines 42-52).

However, Wittwer does not teach a method in which amplification products from the first round are re-amplified by dividing the first-round products into a plurality of aliquots and performing real-time PCR of at least one of the aliquots using at least one primer pair and oligonucleotide probe that was used in the first round of amplification, wherein the probe is complementary to a region of a sequence of interest amplified in the second round of amplification. Wittwer also does not teach a method in which the amplification of step (i) is further carried out in the presence of a reverse transcriptase such that the polymerase chain reaction is reverse-transcription polymerase chain reaction and wherein the one or more polynucleotide sequences is obtained from mRNA derived from the sample. Finally, Wittwer does not teach a method in which an observed efficiency of amplification is greater than 70% or greater than 90%.

Willey teaches a method of multiplex reverse transcription PCR for assessment of gene expression in small biological samples, in which a multiplex reaction is performed in round one using multiple primer pairs for multiple target genes, aliquots of the round one reaction are placed in new reaction tubes with a primer pair specific for one of the desired genes used in round one, and the sample amplified in round two for an additional 35 cycles (paragraph 28, lines 1-7 and paragraph 34, lines 1-15). PCR products from round one can be diluted as much as 100,000-fold and successfully amplified in round two (paragraph 35, lines 1-6). Willey also teaches that although the efficiency of reverse transcription is variable, the representation of one gene to another in the resultant cDNA is not affected and therefore a similar number of cDNA copies will

be obtained that are equivalent to the starting target gene mRNA copies (paragraph 91, lines 10-17).

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Wittwer and Willey since both references teach methods for multiplex PCR amplification of nucleic acid samples. While Wittwer teaches a real-time system using multiple FRET probes to detect and quantify multiple allelic sequences in multiple PCR products, Willey teaches a non-fluorescent RT-PCR assay wherein a sample containing multiple targets is amplified using multiples primers pairs, and then divided into aliquots by dilution of the first amplification products to amplify each target separately. It would have been obvious to one to the ordinary practitioner to use the method of diluting first round amplification products as taught by Willey in the real-time methods of Wittwer to perform a second amplification since rare targets that may not be detected in the first round may be successfully amplified and detected in the second amplification. Thus, an ordinary practitioner would have been motivated to use the methods of Willey using two rounds of amplification in the real-time methods of Wittwer since the methods of Willey provide a means to reduce the amount of cDNA required and reagents consumed by automated and miniaturized platforms, yet maintain the needed sensitivity to detect rare transcripts (Willey, paragraphs 23 and 24). "Thus, using two round of amplification, the same amounts of cDNA and CT mixture that typically are used to obtain one gene expression measurement when only one round of amplification is used can be used to obtain 100,000 gene expression measurements without loss of sensitivity to detect rare

transcripts" (Willey, paragraph 35, lines 6-11). The use of a two-phase amplification system such as that taught by Willey also "provides a method to accurately and efficiently correlate gene expression patterns with clinically relevant phenotypes" (Willey, paragraph 31). Furthermore, Willey teaches that production of cDNA from mRNA is highly efficient, as cDNA copies produced reflect the initial copies of mRNA present (paragraph 91, lines 10-17). Finally, it is notable that Wittwer also teaches a pre-amplification of samples prior to multiplex real-time PCR since the starting sample may be the product of one or more PCR reactions (column 4, lines 11-13 and column 14, lines 60-61) and thus Wittner is also concerned with enriching the sample prior to a second amplification using labeled hybridization probes.

5. Claim 3 is rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer (U.S. Patent No. 6,472,156) in view of Willey et al. (U.S. Patent Pub. No. 2003/0186246) as applied to claims 5, 6, 8, 9, 22, 23, 44 and 45 above, and further in view of Park et al. (U.S. Patent Pub. No. 2004/0146897).

Wittwer and Willey together teach the limitations of claims 5, 6, 8, 9, 22, 23, 44 and 45, as discussed above. However, neither Wittwer nor Willey teach a method in which the one or more polynucleotide sequences comprise a cDNA library.

Park teaches a method for optimizing multiplex PCR by varying reaction conditions such as primer annealing temperature and extension times, and further teaches that the multiplex reactions may be performed on a variety of samples,

including genomic DNA and DNA cloned into vectors in the form of a cDNA library (paragraph 10, lines 1-7, paragraph 29, lines 1-4 and claim 2).

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Wittwer and Willey for multiplex PCR amplification of nucleic acid samples with the methods of Park, who also teaches methods of multiplex PCR and further teaches that the samples used in such multiplex systems may be in variety of different forms, such as blood, genomic DNA or cDNA libraries. Thus, an ordinary practitioner would have been motivated to combine the methods of Wittwer and Willey with those of Park, since Park provides additional types of samples that would be compatible with the multiplex systems of Wittwer and Willey. Furthermore, the methods of Park would be useful for optimizing the multiplex assays of the Wittwer and Willey and thus standardize the methods for detecting multiple targets using miniaturized and fast DNA diagnosis equipment and a variety of sample types (Park, paragraphs 10, 14 and 15).

6. Claim 43 is rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer (U.S. Patent No. 6,472,156) in view of Willey et al. (U.S. Patent Pub. No. 2003/0186246) as applied to claims 5, 6, 8, 9, 22, 23, 44 and 45 above, and further in view of Jansen et al. (U.S. Patent Pub. No. 2005/0175987).

Wittwer and Willey together teach the limitations of claims 5, 6, 8, 9, 22, 23, 44 and 45, as discussed above. However, neither Wittwer nor Willey teach a method in which the amplification is carried out in the presence of uracil N-glycosylase.

Jansen teaches methods for performing real-time multiplex PCR assays for detecting a plurality of HPV genes in the same HPV subtype using multiple fluorophores, and further teaches that the PCR master mix may include dUTP in place of dTTP, and uracil-N-glycosylase (UNG), an enzyme that cleaves uracil-containing nucleic acids (paragraph 13, lines 1-5 and paragraph 152, lines 1-6).

It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine the methods of Wittwer and Willey for multiplex PCR amplification of nucleic acid samples with the methods of Jansen, who also teaches methods of multiplex PCR and further teaches that the PCR assay may be optimized by including dUTP and uracil-N-glycosylase (UNG) in the reaction mixtures. Thus, an ordinary practitioner would have been motivated to combine the methods of Wittwer and Willey with those of Jansen, since Jansen teaches that the use of UNG is an easy means to prevent re-amplification of carryover PCR products in subsequent experiments (Jansen, paragraph 152, lines 5-6) and thus avoid false positive results by contamination of future samples and assays with previously amplified material. Furthermore, UNG is easily inactivated at 50°C to avoid loss of newly amplified samples (paragraph 152, line 3).

Response to Arguments

7. Applicant's arguments filed January 6, 2011 have been fully considered but they are not persuasive.

Applicant argues that the rejection of claims 5, 6, 8, 9, 22, 23, 44 and 45 under 35 U.S.C. § 103(a) as being obvious over Wittwer et al. (U.S. Patent No. 6,472,156) in view of Willey et al. (U.S. Patent Pub. No. 2003/0186246) should be withdrawn since the combination of the references does not teach all the limitations of the claims nor provide the necessary motivation to combine the different methods. In particular, Applicant argues that neither Wittwer nor Willey teach a method to include a plurality of probes in a first round multiplex amplification reaction prior to performing a second round of amplification involving real-time PCR. Applicant argues that the Examiner has failed to describe any "design incentives and other marker forces" that would have provided the motivation to take a highly complicated multiplex amplification reaction, as taught by Willey, and further complicate it by adding oligonucleotide probes, as presently claimed. As Applicant has previously argued, performing such as combination would not have been obvious to one of skill in the art at the time the invention was made since the process would be expected to produce an excess of unwanted side products and would have been considered a wasteful use of expensive-to-manufacture oligonucleotide probes. Applicant maintains that the surprising success of the invention using the claimed methods cannot be drawn from the cited references in the absence of hindsight.

The Examiner asserts that the order of the steps discussed in the rejection above is reversed in Applicant's arguments with regard to base claim 45. Stated another way, The Examiner argues that step (i) is performed by the multiplex real-time PCR methods taught by Wittwer using a plurality of primers and probes, in contrast to using the methods of Willey in step (i), as argued by Applicant. The Examiner then asserts that step (ii) is performed analogous to the teachings of Willey wherein a multiplex reaction is divided into separate PCR reactions, with each separate reaction subjected to PCR using primers for only one of the genes amplified in the first round (Willey, paragraph 28, lines 1-7). Thus, step (i) as taught by Wittwer is a multiplex reaction containing a plurality of both primers and probes, in which products are monitored in real-time (Wittwer, column 17, lines 1-8). Therefore, the potential problems that Applicant argues would not lead one of skill in the art to use such a process, i.e. the production of unwanted non-specific side products and the use of expensive-to-manufacture probes, had already been addressed by Wittwer at the time of the invention to provide a successful multiplex reactions using labeled probes. Willey provides teaching that reactions from a multiplex reaction can be diluted as much as 100,000-fold and re-amplified in separate reactions using just one primer pair used in the multiplex reaction to enable detection of rare transcripts that were not detectable using a single round of amplification (Willey, paragraph 35, lines 1-14). One of skill in the art would recognize that difficult-to-detect targets in the multiplex system of Wittwer could be re-amplified by simply diluting the products and re-amplified using just one specific primer and probe pair used in the initial amplification. Finally, it is notable that Wittwer is also concerned

with enriching the sample prior to a second amplification using labeled hybridization probes, as the reference teaches that starting samples may be provided as previously amplified PCR products prior to multiplex real-time PCR (column 4, lines 11-13 and column 14, lines 60-61). Willey simply provides a different approach to enrich for rare targets, using a second amplification of diluted products after the initial multiplex reaction. Therefore, for all the reasons discussed above, the 103 rejection of claims 5, 6, 8, 9, 22, 23, 44 and 45 over Wittwer in view of Willey is maintained.

Applicant then argues that rejection of dependent claim 3 under 35 U.S.C. § 103(a) as being obvious over Wittwer in view of Willey and further in view of Park et al. (U.S. Patent Pub. No. 2004/0146897) should be withdrawn since Park does not make up for the deficiencies of Wittwer and Willey with respect to base claim 45. As discussed above, the combination of Wittwer and Willey teach the limitations of the base claim and provide the necessary motivation for one of skill in the art to combine such methods. Since the rejection of claim 3 regarding the teachings of Park is not argued separately, the rejection is maintained.

Applicant then argues that rejection of dependent claim 43 under 35 U.S.C. § 103(a) as being obvious over Wittwer in view of Willey and further in view of Jansen et al. (U.S. Patent Pub. No. 2005/0175987) should be withdrawn since Park does not make up for the deficiencies of Wittwer and Willey with respect to base claim 45. As discussed above, the combination of Wittwer and Willey teach the limitations of the base claim and provide the necessary motivation for one of skill in the art to combine

such methods. Since the rejection of claim 43 regarding the teachings of Jansen is not argued separately, the rejection is maintained.

Summary

8. Claims 2, 3, 5, 6, 8, 9, 22, 23 and 43-45 are rejected. No claims are allowable.

Conclusion

9. **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Correspondence

10. Any inquiry concerning this communication or earlier communications from the examiner should be directed to David C. Thomas whose telephone number is 571-272-3320 and whose fax number is 571-273-3320. The examiner can normally be reached on 5 days, 9-5:30.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/David C Thomas/
Examiner, Art Unit 1637

/Kenneth R Horlick/
Primary Examiner, Art Unit 1637